



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁵ : C12N 15/19, C07K 13/00, A61K 37/02	A1	(11) International Publication Number: WO 94/24285 (43) International Publication Date: 27 October 1994 (27.10.94)
(21) International Application Number: PCT/GB94/00822 (22) International Filing Date: 19 April 1994 (19.04.94) (30) Priority Data: 9308060.4 19 April 1993 (19.04.93) GB (71) Applicant (for all designated States except US): CANCER RESEARCH CAMPAIGN TECHNOLOGY LIMITED [GB/GB]; Cambridge House, 6-10 Cambridge Terrace, Regent's Park, London NW1 4JL (GB). (72) Inventors; and (75) Inventors/Applicants (for US only): GRAHAM, Gerard [GB/GB]; Beatson Institute for Cancer Research, Cancer Research Campaign Beatson Laboratories, Garscube Estate, Bearsden, Glasgow G61 1BD (GB). PRAGNELL, Ian [GB/GB]; Beatson Institute for Cancer Research, Cancer Research Campaign Beatson Laboratories, Garscube Estate, Bearsden, Glasgow G61 1BD (GB). (74) Agents: CRESSWELL, Thomas, Anthony et al.; J.A. Kemp & Co., 14 South Square, Gray's Inn, London WC1R 5LX (GB).	(81) Designated States: JP, US, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE). Published With international search report.	
(54) Title: MACROPHAGE INFLAMMATORY PROTEIN VARIANTS		
<p>MIP - 1αc NH₂-----CC-----++ +-----C-----++ +-----C-----++-----COOH</p> <p>(1) NH₂-----CC-----++ +-----C-----++ +-----C-----++-----COOH</p> <p>(2) NH₂-----CC-----++ +-----C-----++ +-----C-----++-----COOH</p> <p>(3) NH₂-----CC-----++ +-----C-----++ +-----C-----++-----COOH</p>		
(57) Abstract <p>The present invention provides a Stem Cell Inhibitor (SCI) protein which comprises at least one amino acid alteration from its native form which protein does not significantly aggregate but which retains substantially unaltered stem cell inhibitory activity. The alteration is preferably a conservative substitution of a charged amino acid residue. Such proteins may be used in treating stem cells in a patient undergoing chemotherapy.</p>		

BEST AVAILABLE COPY

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	GB	United Kingdom	MR	Mauritania
AU	Australia	GE	Georgia	MW	Malawi
BB	Barbados	GN	Guinea	NE	Niger
BE	Belgium	GR	Greece	NL	Netherlands
BF	Burkina Faso	HU	Hungary	NO	Norway
BG	Bulgaria	IE	Ireland	NZ	New Zealand
BJ	Benin	IT	Italy	PL	Poland
BR	Brazil	JP	Japan	PT	Portugal
BY	Belarus	KE	Kenya	RO	Romania
CA	Canada	KG	Kyrgyzstan	RU	Russian Federation
CF	Central African Republic	KP	Democratic People's Republic of Korea	SD	Sudan
CG	Congo	KR	Republic of Korea	SE	Sweden
CH	Switzerland	KZ	Kazakhstan	SI	Slovenia
CI	Côte d'Ivoire	LI	Liechtenstein	SK	Slovakia
CM	Cameroon	LK	Sri Lanka	SN	Senegal
CN	China	LU	Luxembourg	TD	Chad
CS	Czechoslovakia	LV	Latvia	TG	Togo
CZ	Czech Republic	MC	Monaco	TJ	Tajikistan
DE	Germany	MD	Republic of Moldova	TT	Trinidad and Tobago
DK	Denmark	MG	Madagascar	UA	Ukraine
ES	Spain	ML	Mali	US	United States of America
FI	Finland	MN	Mongolia	UZ	Uzbekistan
FR	France			VN	Viet Nam
GA	Gabon				

MACROPHAGE INFLAMMATORY PROTEIN VARIANTS

The present invention relates to variants of stem cell inhibitors.

The treatment of cancer with chemotherapeutic agents is designed to attack and destroy cells which are undergoing division within the body. A side effect of such treatment is thus the destruction of normal cells, particularly the stem cells of the haematopoietic system and the epithelial stem cells which line the scalp and gut. Radiation can also cause similar destruction of such cells.

10 It has been proposed that in order to improve the treatment of cancers by chemotherapy it would be desirable to protect stem cells from cell cycle specific cytotoxic drugs. WO89/10133 discloses a stem cell inhibitor and describes the use of the inhibitor in the treatment of cancers. The inhibitor may be
15 administered to a patient in order to protect stem cells during chemotherapy.

Stem Cell Inhibitor (SCI), also known as MIP1- α is a peptide of about 8kD which forms large self aggregates, the molecular weight of which is dependant upon the concentration of SCI/MIP1- α
20 monomers (Graham et al, 1990, Nature 344;442, Wolpe & Cerami, 1989, FASEB J, 3; 2656). It has been found that SCI/MIP1- α has a native, aggregated molecular weight of about 100kD at 0.1mg/ml in physiological buffers such as PBS. It has been found that diluting SCI/MIP1- α to about 20-100ng/ml or less will bring about
25 disaggregation of this protein.

Human SCI/MIP1- α has been cloned by us (Graham et al (1992), Growth Factors 7;151-160). The cDNA has also been cloned by Nakao et al (1990, Mol. Cell, Biol., 10;3646-58) and called LD78 β . A variant of the cDNA LD78 α was also found, which has a
30 very similar sequence. It differs by only 4 amino acid residues. The human cDNA and protein sequence of the factor cloned by us is shown is Seq. ID No. 1. The first 27 amino acids are a leader sequence. The mature protein starts at residue 28 (ala). The

amino acid sequence of the variant found by Nakao et al is shown as Seq. ID No. 3. The leader sequence of the protein is one amino acid shorter and thus the mature protein starts at residue 27 (ala). The sequence of the murine homologue, upon which we have conducted our work, is also known and is very similar. It can be found for example in Graham et al (1994, J. Biol. Chem., 269; 4974-78).

It has been reported (Mantel et al, 1993, PNAS 90;2232) that monomeric SCI/MIP1- α is more active than the aggregated form in inhibiting in vitro and in vivo stem cell proliferation. In using SCI/MIP1- α in the treatment of humans it would be desirable to administer monomeric protein, not just from an activity point of view but also in order to provide reliable and reproducible formulations. However, it is likely that the low concentrations of SCI/MIP1- α which must be made in order to provide monomeric protein will be too low for use in practice.

We have now surprisingly found that it is possible to obtain SCI/MIP1- α variants which retain substantially the activity of the native protein but which do not form the same large aggregates. These mutants are stable as monomers or as small conglomerates (eg dimers or tetramers) at concentrations many fold higher than native SCI/MIP1- α . Thus for those variants which have activity comparable to native SCI/MIP1- α , the variants may have higher activity in vivo on a unit weight basis.

Accordingly, the present invention provides a Stem Cell Inhibitor protein which comprises at least one amino acid alteration from its native form which does not significantly aggregate but which retains substantially unaltered stem cell inhibitory activity. The protein may comprise either the full length stem cell inhibitor or the mature processed form lacking the leader sequence.

The invention also provides pharmaceutical compositions comprising a stem cell inhibitor according to the invention in combination with a pharmaceutically acceptable carrier or diluent, and optionally other therapeutic ingredients. The

carrier(s) must be "acceptable" in the sense of being compatible with the other ingredients of the formulation and not deleterious to the recipients thereof.

The formulations include those suitable for parenteral (including
5 subcutaneous, intramuscular, intravenous, intraperitoneal, intradermal, intrathecal and epidural) administration. The formulations may conveniently be presented in unit dosage form and may be prepared by any of the methods well known in the art of pharmacy. Such methods include the step of bringing into
10 association the active ingredient with the carrier which constitutes one or more accessory ingredients. In general the formulations are prepared by uniformly and intimately bringing into association the active ingredient with liquid carriers.

Formulations suitable for parenteral administration include
15 aqueous and non-aqueous sterile injection solutions which may contain anti-oxidants, buffers, bacteriostats and solutes which render the formulation isotonic with the blood of the intended recipient; and aqueous and non-aqueous sterile suspensions which may include suspending agents and thickening agents, and
20 liposomes or other microparticulate systems which are designed to target the compound to blood components or one or more organs. Suitable liquid carriers include phosphate buffered saline at a pH of between 7.0 and 8.0, for example 7.4. The formulations may be presented in unit-dose or multi-dose containers, for example
25 sealed ampoules and vials, and may be stored in a freeze-dried (lyophilized) condition requiring only the addition of the sterile liquid carrier, for example water for injections, immediately prior to use.

Preferred unit dosage formulations are those containing a daily
30 dose or unit, daily sub-dose, or an appropriate fraction thereof, of an active ingredient.

Formulations of the SCI/MIP-1 α proteins of the present invention preferably contain from 0.05 to 5 mg/ml of protein, for example 0.1 to 1.0 mg/ml. We have found that the solubility of the
35 variants of the invention do vary although the maximum solubility

of any one particular variant may be determined by simple titration by those of skill in the art.

The invention also provides such proteins and compositions for use in a method of treatment of the human or animal body.

- 5 The invention further provides a method for treating a subject who is to be exposed to an agent capable of killing dividing or cycling stem cells by administering to the subject an effective amount of a protein or composition according to the invention.

The subject may also be treated with a protein or composition
10 according to the invention during or after chemotherapy. In the latter case, this will usually be for a period sufficient to allow clearance of the agent from the body.

The method of treatment according to the invention may be used in the treatment of solid tumours or leukemias. In the case of
15 treatment of leukemias, it is possible to treat a sample of the patients bone marrow which has been removed from the body while the patient is undergoing treatment. The bone marrow is purged of cancer cells in the presence of a protein of composition according to the invention, and the treated marrow reintroduced
20 into the patient.

Although the dose of the variant protein according to the invention will ultimately be at the discretion of the physician, taking into account the nature of the condition being treated and the state of the patient, effective doses may be in the range of
25 from about 10 $\mu\text{g/kg}$ body weight to about 5 mg/kg of variant protein, for example from about 50 to about 1000 $\mu\text{g/kg}$, eg about 500 $\mu\text{g/kg}$.

We have also found that SCI/MIP1- α can act to enhance the expansion of primitive haemopoietic cells in ex vivo cytokine
30 driven stem cell expansion experiments. Thus, variant proteins of the invention may also be used in methods to expand stem cell populations removed from a patient ex vivo wherein such stem

cells are brought into contact with growth factors and the variant proteins of the invention under conditions which allow the growth and expansion in numbers of the cells, prior to reintroduction into the same or another patient. Such a method
5 could be used in bone marrow transplant procedures whereby a limited number of starting cells obtained from a donor are expanded prior to transplantation, or in certain therapies where a sample of bone marrow is removed from a patient prior to treatment and reintroduced following treatment. Such therapies
10 include the treatment of leukemias, or other tumours including solid tumours where damage to the bone marrow may occur. The concentration of the variant proteins required to produce suitable activity will be in the range of from about 1 to about 100 ng/ml, for example from about 10 to about 50 ng/ml.

15 A protein or composition according to the invention may also be used in the treatment of disorders caused by proliferation of stem cells, eg. psoriasis.

A protein according to the invention is preferably a protein which contains at least one change from the native protein
20 resulting in the loss of one or more charges on the protein, eg. by replacement of one or more charged amino acids.

The change may be as a result of a deletion or substitution or insertion. In the case of a deletion or insertion, single base deletions or insertions are generally preferred, in order to
25 retain a structure similar to the native protein. However, deletions or insertions of more than this, eg or 2, 3, 4, 5 or more amino acids are possible. In the case of a substitution, it is preferably a conservative substitution, such as Asp to Asn or Glu to Gln.

30 In addition, fragments of native protein which retain their stem cell inhibitory activity but which exhibit the reduced tendency to aggregate are within the scope of the invention.

Preferably, the change to the protein is in the C-terminal region, eg within the last 20 or even last 10 amino acids. This

may include C-terminal deletions.

More than one change to a native stem cell inhibitor protein may be made. For example, 2, 3, 4 or 5 changes may be made.

Another preferred region of the MIP1 protein which may be altered
5 is the putative heparin binding region between amino acids 68 and
71 of Seq. ID No. 1. We have determined by experimentation and
by comparison of this sequence with known heparin binding regions
that this portion of MIP1 has heparin binding activity. Thus
suitable amino acids which may be altered in accordance with the
10 invention include one, two or three of 68(lys), 69(arg) and
71(arg). Such alterations may be made, if desired with an
alteration to the c-terminal region of the MIP1 protein as
described above.

Preferred stem cell inhibitor proteins of the invention are those
15 based upon the human protein of Seq. ID. 2 or that of Seq. ID 3.
Also preferred are the mature forms of such proteins, ie. from
residues 28 onwards.

Particular amino acids which may be altered in the protein
sequence of Seq. ID No.2 or Seq. ID No. 3 include alterations at
20 any positively charged residue, eg. lys or arg, and/or at any
negatively charged residue, eg asp or glu. The residues of Seq.
ID. No. 2 which may be altered thus include: 29(asp), 41(arg),
50(asp), 53(glu), 60(lys), 68(lys), 69(arg), 71(arg), 76(asp),
79(glu), 80(glu), 84(lys), 87(asp) or 90(glu). The changes made
25 to these positions may be as described above.

Combinations of changes which may be made include changing the
final 2, 3, 4, 5 or 6 charged residues of the stem cell
inhibitor. In the case of the human protein, this results in a
protein which corresponds to the native protein except for
30 changes at position 90 and/or one or more of positions 76, 79,
80, 84 or 88. Preferably, all the changes are single amino acid
substitutions. Preferably, all such substitutions are
conservative changes.

- 7 -

Proteins according to the invention may be made by any means available in the art. In the examples which follow, we have made modified stem cell inhibitory proteins by site directed mutagenesis using PCR primers of the murine SCI cDNA, followed by
5 expression of the modified cDNA in a vector in a host cell to produce the protein. The protein may be recovered from the host cell using protein purification techniques known per se. Analogous methods may be used to make modified human or other primate SCI. The murine cDNA may be obtained for example by
10 reference to the methods disclosed in WO89/10133 or by reference to the published literature. Human cDNA may also be obtained by reference to the published literature or cloned using probes based on all or part of the DNA sequence of Seq. ID No. 1 to identify SCI cDNA in a cDNA library made from cells expressing
15 SCI RNA.

Accordingly, the present invention also provides a method for making a protein according to the invention which comprises:
(i) modifying a DNA sequence coding for SCI protein in order to introduce at least one change which causes a change in the amino
20 acid sequence of the SCI protein;
(ii) expressing said DNA, operably linked to a promoter, in a vector in a host cell compatible with said promoter; and
(iii) recovering said protein.

The DNA may be modified by site directed mutagenesis as mentioned
25 above or described in the examples, to obtain insertions, deletions or substitutions in the amino acid sequence.

The vector may contain one or more selectable marker genes, for example an ampicillin resistance gene in the case of a bacterial plasmid or a neomycin resistance gene for a mammalian vector.

30 A further embodiment of the invention provides host cells transformed or transfected with the vectors for the replication and expression of DNA produced as described above, including the DNA Seq. ID No. 1 modified as mentioned above. The cells will be chosen to be compatible with the vector and may for example be
35 bacterial, yeast, insect or mammalian.

The invention also provides monoclonal or polyclonal antibodies to a peptide according to the invention which is directed to an epitope containing an alteration of the native SCI. The invention further provides a process for the production of such 5 monoclonal or polyclonal antibodies. Monoclonal antibodies may be prepared by conventional hybridoma technology using the proteins or peptide fragments thereof, as an immunogen. Polyclonal antibodies may also be prepared by conventional means which comprise inoculating a host animal, for example a rat or a 10 rabbit, with a peptide of the invention and recovering immune serum.

In either case, antibodies which recognise altered epitopes may be identified by screening them with native SCI and the altered SCI to which the antibody was raised and identifying an antibody 15 which recognises only the altered SCI.

The following examples illustrate the invention.

Example 1

Figure 1 shows a schematic representation of murine SCI/MIP1- α indicating the position of charged amino acids. A series of 20 altered proteins (1) - (3) were made using PCR primers on cDNA encoding the protein together with a wild type 5' primer. The altered proteins all contained conservative changes, ie. glutamate to glutamine and/or aspartate to asparagine. The primers used are as follows:

25 Variant 1:

5' TC AGG AAT TCA GGC ATT CAG TTG CAG GTC 3' (SEQ ID NO. 4).

This alters the C-terminal end of the murine MIP1- α protein from: VQ EYITDLELNA (SEQ ID NO. 5) to VQ EYITDLQLNA (SEQ ID NO.6).

Variant 2:

30 5' TC AGG AAT TCA GGC ATT CAG TTG CAG GTT AGT GAT 3' (SEQ ID NO.7)
which alters Seq. ID No. 5 to VQ EYITNLQLNA (SEQ ID NO.8).

Variant 3:

5' TC AGG AAT TCA GGC ATT CAG TTG CAG GTT AGT GAT GTA TTG
 TTG GAC 3' (SEQ ID NO. 9)
 which alters Seq. ID No. 5 to VQQYITNLQLNA (SEQ ID NO.10)

The varied cDNA molecules were ligated into a fusion protein
 5 expression vector and the altered proteins were produced. The
 native protein together with the three altered proteins were
 analysed by chromatographic techniques and the molecular weights
 of each estimated.

The estimates were as follows:

10 Native protein	100-150 kD
Protein (1)	35 kD
Protein (2)	18 kD
Protein (3)	8 kD

Protein (1) thus appears to exist as a tetramer, protein (2) as
 15 a dimer and protein (3) as a monomer under conditions in which
 native MIP1- α exists as an aggregated protein.

The above proteins were assessed for bioactivity using standard
 techniques (Pragnell et al Blood, 1988, 72; 196 and Lorimore et
 al, 1990, Leukaemia Research 14; 481) and found to be bioactive.

20 Example 2

Two 3' (carboxy terminus) primers were synthesised with the
 following sequences:

5' GTA CGT GGA TCC TCA GGC ACT CAG CTG CAG GTT GCT GAC ATA TTG
 CTG GAC 3' (SEQ ID NO. 11)

25 and

5' GTA CGT GGA TCC TCA GGC ACT CAG CTG CAG GTT GCT GAC ATA TTG
 CTG GAC CCA CTG CTC ACT 3' (SEQ ID NO. 12).

A Bam H1 recognition site is underlined.

The primer of Seq. ID No. 11 encodes amino acids 82 to 93 of Seq.
 30 ID No. 1 but alters the lysine at position 84 (84(lys)) to
 glutamine (gln), 88(asp) to asn, and 90(glu) to gln.

The primer of Seq. ID. No. 12 encodes to amino acids 78 to 93 of Seq. ID No. 1 but contains the three changes described above for Seq. ID No. 11 and also a further change, 80(glu) to gln.

To produce the human variants incorporating the above changes the 5 above primers are each used with an amino terminal primer of Seq. ID No. 13:

5' GAC GGC CAT GGC TGA CAC GCC GAC CGC CTG C 3' (SEQ ID NO. 13)
which encodes amino acids 28-35 of Seq. ID No. 1. An NcoI recognition site is underlined. This corresponds to the start of
10 the mature SCI/MIP-1 protein.

The primers are used in a PCR to provide full length clones encoding variants incorporating the changes described above, and the variant clones introduced into an expression vector to provide disaggregated variant proteins of the invention.

15 The variants are tested in a similar manner as described above for activity.

Example 3

A internal primer which encodes a central portion of the murine MIP1- α protein was designed, incorporating changes which cause
20 point mutations in two of the three positively charged residues between the third and fourth cysteine residues shown in Figure 1(a). The primer is of the sequence:

5' CGT CTA GAC GGC CAA CGA CAA TCA GTC CTT 3' (SEQ ID NO. 14)
which alters the murine sequence:

25 FLTKRNRQIC (SEQ ID NO. 15) to FLTNSNRQIC (SEQ ID NO. 16).

The mutagenesis was done in two halves using this primer and the wild type amino terminal primer and a complementary primer was used with the wild type carboxy terminal primer. The two reaction products were then mixed and the full length molecule produced
30 using the wild type amino and carboxy terminal primers. The variant is also tested for activity.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:

(A) NAME: Cancer Research Campaign Technology Limited
 (B) STREET: 6-10 Cambridge House
 (C) CITY: London
 (E) COUNTRY: GB
 (F) POSTAL CODE (ZIP): NW1 4JL

(i) APPLICANT:

(A) NAME: Graham, Gerard
 (B) STREET: Beatson Laboratories, Garscube Estate
 (C) CITY: Glasgow
 (E) COUNTRY: GB
 (F) POSTAL CODE (ZIP): G61 1BD

(i) APPLICANT:

(A) NAME: Pragnell, Ian
 (B) STREET: Beatson Laboratories, Garscube Estate
 (C) CITY: Glasgow
 (E) COUNTRY: GB
 (F) POSTAL CODE (ZIP): G61 1BD

(ii) TITLE OF INVENTION: Stem Cell Inhibitor

(iii) NUMBER OF SEQUENCES: 16

(iv) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Floppy disk
 (B) COMPUTER: IBM PC compatible
 (C) OPERATING SYSTEM: PC-DOS/MS-DOS
 (D) SOFTWARE: PatentIn Release #1.0, Version #1.25 (EPO)

(v) CURRENT APPLICATION DATA:

APPLICATION NUMBER:

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 282 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: double
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:

(A) NAME/KEY: CDS
 (B) LOCATION: 1..282

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

ATG CAG GTC TCC ACT GCT GCC CTT GCC GTC CTC CTC TGC ACC ATG GCT	48
Met Gln Val Ser Thr Ala Ala Leu Ala Val Leu Leu Cys Thr Met Ala	
1 5 10 15	
CTC TGC AAC CAG GTC CTC TCT GCA CCA CTT GCT GCT GAC ACG CCG ACC	96
Leu Cys Asn Gln Val Leu Ser Ala Pro Leu Ala Ala Asp Thr Pro Thr	
20 25 30	
GCC TGC TGC TTC AGC TAC ACC TCC CGA CAG ATT CCA CAG AAT TTC ATA	144
Ala Cys Cys Phe Ser Tyr Thr Ser Arg Gln Ile Pro Gln Asn Phe Ile	
35 40 45	

- 12 -

```

GCT GAC TAC TTT GAG ACG AGC AGC CAG TGC TCC AAG CCC AGT GTC ATC.      192
Ala Asp Tyr Phe Glu Thr Ser Ser Gln Cys Ser Lys Pro Ser Val Ile
   50                               55                               60

TTC CTA ACC AAG AGA GGC CGG CAG GTC TGT GCT GAC CCC AGT GAG GAG      240
Phe Leu Thr Lys Arg Gly Arg Gln Val Cys Ala Asp Pro Ser Glu Glu
   65                               70                               75                               80

TGG GTC CAG AAA TAC GTC AGT GAC CTG GAG CTG AGT GCC TGA              282
Trp Val Gln Lys Tyr Val Ser Asp Leu Glu Leu Ser Ala *
   85                               90

```

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 93 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

```

Met Gln Val Ser Thr Ala Ala Leu Ala Val Leu Leu Cys Thr Met Ala
  1                               5                               10                               15

Leu Cys Asn Gln Val Leu Ser Ala Pro Leu Ala Ala Asp Thr Pro Thr
  20                               25                               30

Ala Cys Cys Phe Ser Tyr Thr Ser Arg Gln Ile Pro Gln Asn Phe Ile
  35                               40                               45

Ala Asp Tyr Phe Glu Thr Ser Ser Gln Cys Ser Lys Pro Ser Val Ile
  50                               55                               60

Phe Leu Thr Lys Arg Gly Arg Gln Val Cys Ala Asp Pro Ser Glu Glu
  65                               70                               75                               80

Trp Val Gln Lys Tyr Val Ser Asp Leu Glu Leu Ser Ala
  85                               90

```

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 92 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

```

Met Gln Val Ser Thr Ala Ala Leu Ala Val Leu Leu Cys Thr Met Ala
  1                               5                               10                               15

Leu Cys Asn Gln Phe Ser Ala Ser Leu Ala Ala Asp Thr Pro Thr Ala
  20                               25                               30

Cys Cys Phe Ser Tyr Thr Ser Arg Gln Ile Pro Gln Asn Phe Ile Ala
  35                               40                               45

Asp Tyr Phe Glu Thr Ser Ser Gln Cys Ser Lys Pro Gly Val Ile Phe
  50                               55                               60

Leu Thr Lys Arg Ser Arg Gln Val Cys Ala Asp Pro Ser Glu Glu Trp
  65                               70                               75                               80

Val Gln Lys Tyr Val Ser Asp Leu Glu Leu Ser Ala
  85                               90

```

(2) INFORMATION FOR SEQ ID NO:4:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 29 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

TCAGGAATTC AGGCATTCAG TTGCAGGTC

29

(2) INFORMATION FOR SEQ ID NO: 5:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 12 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

Val	Gln	Glu	Tyr	Ile	Thr	Asp	Leu	Glu	Leu	Asn	Ala
1				5					10		

(2) INFORMATION FOR SEQ ID NO: 6:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 12 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

Val	Gln	Glu	Tyr	Ile	Thr	Asp	Leu	Gln	Leu	Asn	Ala
1				5					10		

(2) INFORMATION FOR SEQ ID NO:7:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 35 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

TCAGGAATTC AGGCATTCAG TTGCAGGTTA GTGAT

35

(2) INFORMATION FOR SEQ ID NO: 8:

- (i) SEQUENCE CHARACTERISTICS:

- 14 -

- (A) LENGTH: 12 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

Val	Gln	Glu	Tyr	Ile	Thr	Asn	Leu	Gln	Leu	Asn	Gln
1				5					10		

(2) INFORMATION FOR SEQ ID NO:9:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 47 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

TCAGGAATTC AGGCATTCAG TTGCAGGTTA GTGATGTATT GTTGGAC

47

(2) INFORMATION FOR SEQ ID NO: 10:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 12 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

Val	Gln	Gln	Tyr	Ile	Thr	Asn	Leu	Gln	Leu	Asn	Ala
1				5					10		

(2) INFORMATION FOR SEQ ID NO: 11:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 51 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

GTACGTGGAT CCTCAGGCAC TCAGCTGCAG GTTGCTGACA TATTGCTGGA C

51

(2) INFORMATION FOR SEQ ID NO:12:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 63 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

GTACGTGGAT CCTCAGGCAC TCAGCTGCAG GTTGCTGACA TATTGCTGGA CCCACTGCTC 60
ACT 63

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 31 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

GACGGCCATG GCTGACACGC CGACCGCCTG C 31

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

CGTCTAGACG GCCAACGACA ATCAGTCCTT 30

(2) INFORMATION FOR SEQ ID NO: 15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 15:

Phe Leu Thr Lys Arg Asn Arg Gln Ile Cys
1 5 10

(2) INFORMATION FOR SEQ ID NO: 16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 16:

Phe Leu Thr Asn Ser Asn Arg Gln Ile Cys
1 5 10

CLAIMS

1. A Stem Cell Inhibitor (SCI) protein which comprises at least one amino acid alteration from its native form which protein does not significantly aggregate but which retains substantially unaltered stem cell inhibitory activity.
2. A protein according to claim 1 which exists as a tetramer, dimer or monomer under conditions in which the native protein exists as an aggregate.
3. A protein according to claim 1 or 2 wherein the alteration is an amino acid substitution.
4. A protein according to claim 3 wherein the amino acid substitution results in the loss of a charged amino acid.
5. A protein according to claim 3 or 4 wherein the substitution is a conservative substitution.
6. A protein according to claim 5 wherein the substitution is of Asp to Asn or Glu to Gln.
7. A protein according to any one of claims 1 to 6 which is a mature stem cell inhibitor.
8. A protein according to any one of claims 1 to 7 wherein the native form of protein is human stem cell inhibitor.
9. A protein according to claim 8 wherein the amino acid alteration is at one or more of 29(asp), 41(arg), 50(asp), 53(glu), 60(lys), 68(lys), 69(arg), 71(arg), 76(asp), 79(glu), 80(glu), 84(lys), 87(asp) or 90(glu).
10. A protein according to any one of the preceding claims which contains 2 or 3 amino acid alterations.
11. A pharmaceutical composition comprising a protein according to any one of claims 1 to 10 in combination with a carrier

or diluent.

12. A protein according to any one of claims 1 to 10 or a composition according to claim 11 for use in a method of treatment of the human or animal body.
13. A method for treating a subject who is to be exposed to an agent capable of killing dividing or cycling stem cells by administering to the subject an effective amount of a protein as defined in any one of claims 1 to 10 or a composition according to claim 11.
14. A method for making a protein as defined in any one of claims 1 to 10 which comprises:
 - (i) modifying a DNA sequence coding for SCI protein in order to introduce at least one change which causes a change in the amino acid sequence of the SCI protein;
 - (ii) expressing said DNA, operably linked to a promoter, in a vector in a host cell compatible with said promoter; and
 - (iii) recovering said protein.

INTERNATIONAL SEARCH REPORT

Intern. Application No

PCT/GB 94/00822

A. CLASSIFICATION OF SUBJECT MATTER
 IPC 5 C12N15/19 C07K13/00 A61K37/02

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
 IPC 5 C12N C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	WO,A,93 13206 (BRITISH BIO-TECHNOLOGY LTD.; GB) 8 July 1993 see the whole document ---	1-14
X	GROWTH FACTORS vol. 7, no. 2, 1992 pages 151 - 160 GRAHAM, G.J. ET AL.; 'Purification and biochemical characterization of human and murine stem cell inhibitors (SCI).' see page 158, column 1, line 28 - page 159, column 1, line 2 ---	1-3,5,7, 8,10-13
X	WO,A,91 04274 (GENETICS INSTITUTE, INC.; US) 4 April 1991 ---	1,3,5,7, 8,10-13
Y	see the whole document ---	2
	--- -/--	

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents:

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the international filing date
- *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed

T later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

X document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

Y document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

A document member of the same patent family

Date of the actual completion of the international search

11 July 1994

Date of mailing of the international search report

14. 07. 94

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
 NL - 2280 HV Rijswijk
 Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
 Fax: (+31-70) 340-3016

Authorized officer

Nauche, S

INTERNATIONAL SEARCH REPORT

Int. Application No
PCT/GB 94/00822

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA. vol. 90 , 15 March 1993 , WASHINGTON US pages 2232 - 2236 MANTEL, C. ET AL.; 'Polymerization of murine macrophage inflammatory protein 1 alpha inactivates its myelosuppressive effects in vitro : The active form is a monomer.' see the whole document ---	2
X	WO,A,92 05198 (CHIRON CORPORATION) 2 April 1992	1,3,5,7, 8,10-13
Y	see the whole document ---	2
P,X	JOURNAL OF BIOLOGICAL CHEMISTRY. vol. 269, no. 7 , 18 February 1994 , BALTIMORE US pages 4974 - 4978 GRAHAM GJ;MACKENZIE J;LOWE S;TSANG ML;WEATHERBEE JA;ISSACSON A;MEDICHERLA J;FANG F;WILKINSON PC;PRAGNELL IB; 'Aggregation of the chemokine MIP-1 alpha is a dynamic and reversible phenomenon. Biochemical and biological analyses.' see the whole document -----	1-14

INTERNATIONAL SEARCH REPORT

International application No.

PCT/GB94/00822

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Remark : Although claim 13 is directed to a method of treatment of the human/animal body as well as diagnostic methods (Rule 39.1(iv)PCT) the search has been carried out and based on the alleged effects of the compound/composition.
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

information on patent family members

International Application No

PCT/GB 94/00822

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO-A-9313206	08-07-93	AU-B- 3260493	28-07-93
WO-A-9104274	04-04-91	CA-A- 2064558	26-03-91
		EP-A- 0494268	15-07-92
		JP-T- 5502443	28-04-93
WO-A-9205198	02-04-92	CA-A- 2091266	15-03-92
		EP-A- 0548214	30-06-93
		JP-T- 6503710	28-04-94

**This Page is Inserted by IFW Indexing and Scanning
Operations and is not part of the Official Record**

BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images include but are not limited to the items checked:

- ☐ BLACK BORDERS
- ☐ IMAGE CUT OFF AT TOP, BOTTOM OR SIDES
- ☒ FADED TEXT OR DRAWING
- ☒ BLURRED OR ILLEGIBLE TEXT OR DRAWING
- ☐ SKEWED/SLANTED IMAGES
- ☐ COLOR OR BLACK AND WHITE PHOTOGRAPHS
- ☐ GRAY SCALE DOCUMENTS
- ☐ LINES OR MARKS ON ORIGINAL DOCUMENT
- ☐ REFERENCE(S) OR EXHIBIT(S) SUBMITTED ARE POOR QUALITY
- ☐ OTHER: _____

IMAGES ARE BEST AVAILABLE COPY.

As rescanning these documents will not correct the image problems checked, please do not report these problems to the IFW Image Problem Mailbox.